

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Itzhak Bentwich

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Examiner: WOLLENBERGER, LOUIS V

Title: SMALL MOLECULES MODULATING
ACTIVITY OF MICRO RNA
OLIGONULEOTIDES AND MICRO
RNA TARGETS AND USES THEREOF

DECLARATION OF AYELET CHAJUT, PH.D.

Dear Sir:

I, Ayelet Chajut, Ph.D., hereby declare as follows:

1. I am the Executive Vice President, R&D at Rosetta Genomics, Ltd. ("Rosetta"). A true and correct copy of my Curriculum Vitae is attached to this declaration as Exhibit A.

2. I have 22 years of experience designing and performing experiments in the field of molecular biology, 2.5 of which were related to miRNA biology. I have also worked in the biotechnology for 10 years.

3. As a result of my work as Executive Vice President, R&D and experience in the field of molecular biology, I supervised and conducted the experiments to validate miRNA hsa-miR-494 (SEQ ID NO: 7002375) binding to its target transcript MAPKAPK2 (SEQ ID NO: 5210247, RefSeq NM_004759), which are summarized in Appendix A of the reply filed August 1, 2008.

4. Specifically, the experiments entailed transfecting human Hep3B cells with an anti-sense oligonucleotide ("ASO") that specifically binds to hsa-miR-494, and measuring the resulting level of target MAPKAPK2 mRNA as compared to the level of target in cells transfected with a control oligonucleotide that does not bind the miRNA.

5. Hep3B cells from the American Type Culture Collection (Rockville, MD) were plated in 6-well plates 24 hours prior to transfection.

6. Cells were transfected with the specific anti-sense oligonucleotides (ASOs) to miR-494 for 24 and 48 hours using Oligofectamine Invitrogen (Carlsbad, CA). Control cells were either transfected with negative control ASO or not transfected at all. All sets of cells were done in duplicate.

7. After transfection, RNA was isolated and mRNA of MAPKAPK2 was quantified using the specific primers listed below by SYBR qRT-PCR method (Applied Biosystems). mRNA of the house keeping gene TBP was also quantified by SYBR qRT-PCR using the primers for TBP listed in the table below. Total RNA was isolated by EZ-RNA II kit (Biological Industries) 24 and 48 hours after transfection. 1 µg of total RNA was reverse transcribed using Superscript II. After reverse transcription, 10ng of cDNA was used for qRT-PCR reaction.

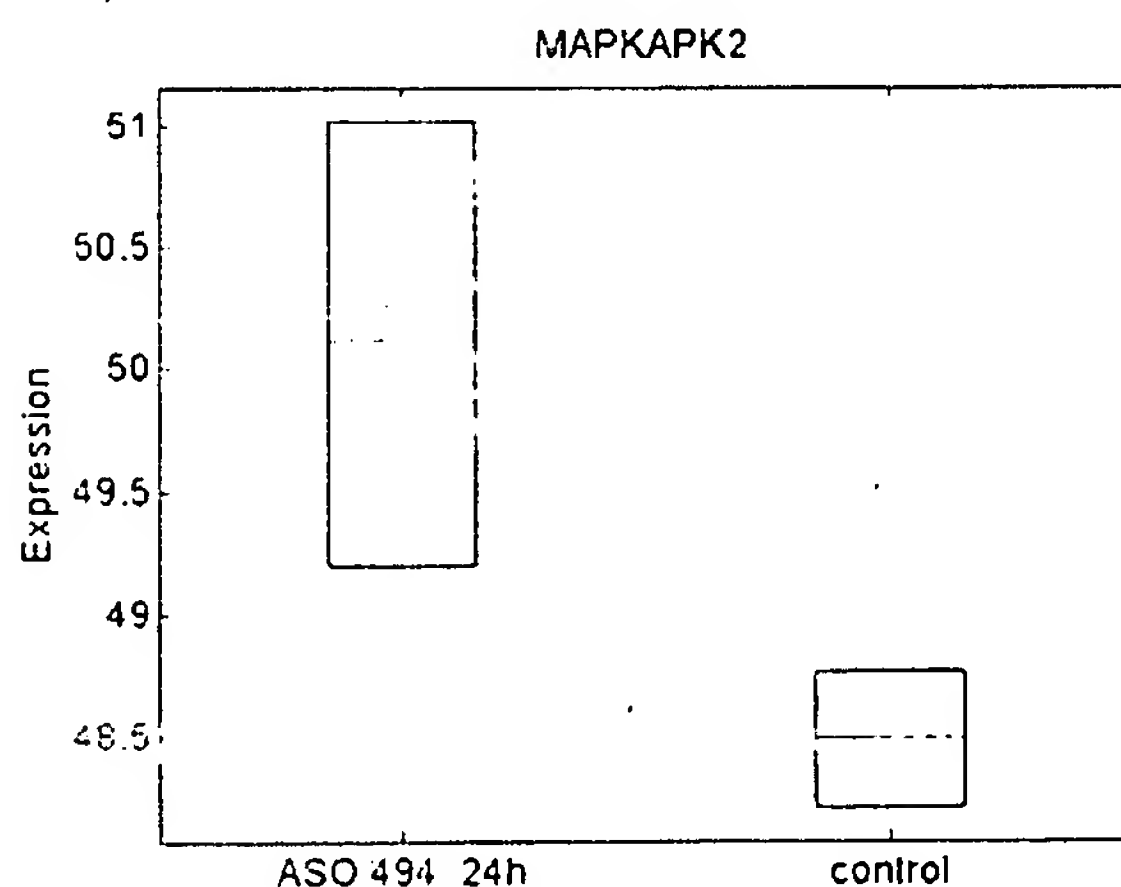
mRNA was quantified by qRT-PCR SYBR Green method (Applied Biosystems) using 7500 Fast Real time PCR. Each test was done in triplicate.

miRNA	Target	ASO for miRNA	Primers for Target	
			Fwd	Rev
hsa-miR-494	MAPKAPK2	GAGGTTTCCCGTGTATGTTTCA	ATCACCCCAACAACTCAGC	TTCCCCCATAACAAAAGACG
house keeping	TBP		TATAATCCCAAGCGGTTTGC	CACAGCTCCCCACCATATTC

8. Measuring the amount of initial mRNA was based on the observation that the amount of cDNA generated from the mRNA doubles with every cycle of PCR. Therefore, after N cycles, there is 2^N times as much. The initial amount of mRNA was quantified by measuring the cycle number at which the increase in fluorescence (and thus the amount of cDNA) was exponential. A threshold at this level of fluorescence was set. This cycle at this point is indicated as the cycle threshold, or Ct. To compare the differences in quantity between a specific mRNA in two different samples, the Ct was calculated in each of the samples, and the delta Ct (ΔCt) was calculated. The fold-change between the amount of mRNA in the two samples was represented by $2^{\Delta Ct}$.

9. The statistical method used to analyze the data was a t-test (two-sided unpaired t-test) between the negative control and the treated samples. Normalization was done by subtracting the Ct value of a housekeeping gene-TBP. Ct values were determined using a default threshold of 0.2 in the 7500 Fast Real time PCR system (Applied Biosystems), and Ct values were normalized to the housekeeping gene TBP.

10. Results of MAPKAPK2 expression with and without ASO to miR-494 are shown below:



- fold change for miR-494 is: 3 (i.e., $2^{\Delta Ct} = 2^{(50.10 - 48.49)}$)

11. The above plot shows that the mRNA of MAPKAPK2 in cells transfected with an ASO that targets hsa-miR-494 is increased approximately 3-fold as compared to irrelevant-ASO transfected control cells.

12. I solemnly declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 35 U.S.C. § 1001, and may jeopardize the validity of the application or any patent issuing thereon.

Dated: 23.09.08

By:

A. Chajut
Ayelet Chajut, Ph.D.

Exhibit A

CURRICULUM VITAE

AYELET CHAJUT

PERSONAL

Name: Ayelet Chajut
Date of Birth: 5th August, 1962
Place of Birth: Israel
Family Status: Married + 2
Military Service: 1980-1982
Phone: 03-5401981, 052-4287229
e. mail address: mailto:ayelet_ch@rosettagenomics.com

PROFESSIONAL EXPERIENCE

- 2007- Executive Vice President R&D, Head Molecular Biology, at Rosetta-Genomics.
- 2006- 2007 Vice President Therapeutics, at Rosetta-Genomics.
In this capacity, I am responsible for the development of new drugs based on microRNAs.
- 2005-2006 Director of Science & Technology at Quantomix, Ltd.
In this capacity I am responsible for development of biological applications of the WETSEM technology, mainly in the field of metabolic disorders focusing on the drug development and diagnostic areas, collaborations with academia and pharmaceutical companies.
- 2003-2005 Vice President Research, at Quark Biotech, Inc.
In this capacity, in addition to my previous tasks, I was responsible to the drug discovery units including: Protein expression and purification, bioassay development. Chemical screening, data analysis, hits selection and validation.
- 2002-2003 Senior director of Target Discovery and Validation, at Quark Biotech, Inc
In this capacity, in addition to my previous tasks, I was responsible to target gene validation processes in 5 different pathology-related research teams
- 2000-2002 Director of Target Discovery, at Quark Biotech, Inc
In this capacity I headed the multidisciplinary candidate genes selection committee responsible for nominating and selecting the genes that QBI

should focus research and development efforts on. Responsible for Gene-discovery process units (RNA, cDNA libraries, microarray printing, bioinformatic and data analysis).

- 1998-2000 Senior scientist, in charge of "Stem Cells" research at Quark Biotech. In this capacity I designed a robust gene discovery program aimed at elucidating the mechanisms of pluripotency of Embryonic & Hematopoietic stem cells and identification of new targets. I was responsible for carrying out these plans by managing the internal research efforts as well as collaborations with several leading researchers in the field.
- 1989-1994 Laboratory instructor and tutor of 3rd year medical students, Department of Microbiology, Faculty of Medicine, Tel-Aviv University
- 1993-1999 Managing the "Virology" course in the Open University of Israel, Both from the academic aspect and the administrative aspect.
- 1997-1998 Project manager, Orit – technological R&D center Ltd, Ariel, Israel.

EDUCATION:

- 1994-1997 Post Doctoral studies in the Laboratory of Prof. Sara Lavi, Department of Cell Research and Immunology, Faculty of Life Sciences, Tel-Aviv University, Tel-Aviv. Main study: Molecular and biochemical characterization of protein phosphatase 2C (PP2C) in eukaryotic cells; Identification of a putative new cell cycle regulator.
- 1989-1994 Studies towards Ph.D. degree in the Laboratory of Prof. Abraham Yaniv and Prof. Arnona Gazit in the Department of Human Microbiology, Sackler School of Medicine, Tel-Aviv University, Tel-Aviv. Thesis: "Lymphoproliferative disease virus of turkeys Studies of oncogenetic mechanism".
- 1988-1989 Studies in the Department of Human Microbiology, Sackler School of Medicine, Tel-Aviv University, Tel-Aviv. M.Sc. degree (*summa cum laude*).
- 1983-1986 Studies in the Faculty of Agriculture, Hebrew University, Jerusalem. B.Sc. degree (*cum laude*).

RESEARCH EXPERIENCE

Molecular biology, Protein analysis, Cell culture, in vivo models, Bioinformatics, Microarray design and result analysis,. Bioassay development, HTS screening.

Publications

Articles:

1. Gak, E., Yaniv, A., Chajut, A., Ianconescu, M., Tronick, S.R. and Gazit, A. 1989. *Molecular cloning of an oncogenic replication - competent virus that causes lymphoproliferative disease in turkeys*. J. Virol. 63: 2877 - 2880.
2. Chajut, A., Yaniv, A., Avivi, L., Bar-am, I., Tronick, S.R. and Gazit, A. 1990. *A novel approach for establishing common or random integration loci for retroviral genomes*. Nucleic Acid Res. 15: 4299.
3. Chajut, A., Sarid, R., Gak, E., Yaniv, A., Garry, Tronick, S.R. and Gazit, A. 1992. *The lymphoproliferative disease virus of turkeys is a representative of a distinct class within the retroviridae, evolutionary related to the avian sarcoma- leukemia viruses*. Gene 122: 349 - 354.
4. Sarid, R., Chajut, A., Malkinson, M., Tronick, S.R., Gazit, A. and Yaniv, A. 1994. *Diagnostic test for lymphoproliferative disease virus of turkeys, using the polymerase chain reaction*. Am. J. Vet. Res. 55: 769 - 772.
5. Sarid, R., Chajut, A., Gak, E., Oroszlan, S., Tronick, S.R., Yaniv, A. and Gazit, A. 1994. *Nucleotide sequence and genome organization of a biologically active provirus of the lymphoproliferative disease virus of turkeys*. Virology 204: 648 - 691.
6. Yaniv, A., Sarid, R., Chajut, A., Gak, E., Altstock, R., Tronick, S.R. and Gazit, A. 1995. *The lymphoproliferative disease virus (LPDV) of turkeys*. Isr. J. Veter. Med. 50: 87-95.
7. Chajut, A., Gazit, A. and Yaniv, A. 1996. *The turkey c-rap1A proto-oncogene is expressed via two distinct promoters*. Gene 177: 7-10.
8. Seroussi E, Shani N, Ben-Meir D, Chajut A, Divinski I, Faier S, Gery S, Karby S, Kariv-Inbal Z, Sella O, Smorodinsky NI and Lavi S. 2001. *Uniquely conserved non-translated regions are involved in generation of the two major transcripts of protein phosphatase 2Cbeta*. J Mol Biol. 312:439-51.
9. Shoshani T, Faerman A, Mett I, Zelin E, Tenne T, Gorodin S, Moshel Y, Elbaz S, Budanov A, Chajut A, Kalinski H, Kamer I, Rozen A, Mor O, Keshet E, Leshkowitz D, Einat P, Skaliter R. and Feinstein E. 2002. *Identification of a novel hypoxia-inducible factor 1-responsive gene, RTP801, involved in apoptosis*. Mol Cell Biol. 22:: 2283-93.

10. Budanov AV, Shoshani T, Faerman A, Zelin E, Kamer I, Kalinski H, Gorodin S, Fishman A, Chajut A, Einat P, Skaliter R, Gudkov AV, Chumakov PM and Feinstein E. 2002. *Identification of a novel stress-responsive gene Hi95 involved in regulation of cell viability*. Oncogene. 21: 6017-31.
11. Rosenfeld N, Aharonov R, Meiri E, Rosenwald S, Spector Y, Zepeniuk M, Benjamin H, Shabes N, Tabak S, Levy A, Lebanony D, Goren Y, Silberschein E, Targan N, Ben-Ari A, Gilad S, Sion-Vardy N, Tobar A, Feinmesser M, Kharenko O, Nativ O, Nass D, Perelman M, Yosepovich A, Shalmon B, Polak-Charcon S, Fridman E, Avniel A, Bentwich I, Bentwich Z, Cohen D, Chajut A, Barshack I. 2008. *MicroRNAs accurately identify cancer tissue origin*. Nat Biotechnol. 26:462-9.
12. Gilad S, Meiri E, Yogev Y, Benjamin S, Lebanony D, Yerushalmi N, Benjamin H, Kushnir M, Cholak H, Melamed N, Bentwich Z, Hod M, Goren Y and Chajut A. 2008. *Serum microRNAs are promising novel biomarkers*. PLoS ONE. 5:e3148.

Patents:

1. Chajut, A. 20032. Methods of using colony stimulating factors in the treatment of tissue damage and ischemia. Patent N. US 20020198150
2. Chajut, A., Levinson M. and Skaliter R. 2003. 76A11 polypeptide and uses thereof. Patent N. US 20030157111
3. Byk T. and Chajut, A. 2004. Human protein sFRP1 and therapeutic use for induction of stem cell proliferation. Patent N. US 2004265995.
4. Byk T. Chajut, A. and Visser J 2004. Ctla-2 and uses thereof in the induction of stem cells. Patent N. US 200411340

Chapters in books:

1. Yaniv, A., Sarid, R., Chajut, A., Gak, E., Altstock, R., Smythers, G.W., Tronick, S.R. and Gazit, A. 1992. The lymphoproliferative disease virus (LPDV) of turkeys: an acute retrovirus lacking an oncogene. p. 163-175. In: Frensdorff, A. (ed.), Frontiers in cancer research. "Ramot" Publ. Tel-Aviv University.